

Specific IgE without clinical allergy

Over the past decades, the importance and quality of in vitro tests for the diagnosis of type I allergies has gradually been increasing. Semi-automated or completely automated systems have conquered the market with highly sensitive tests, enabling detection of nanogram quantities of specific IgE. Unfortunately, an in vitro test response cannot always automatically be translated into a clear clinical diagnosis. In particular, tests for food allergy are known to have poor predictive value. False-negative test responses can usually simply be explained by lability of major food allergens in food extracts. A well-known example of a labile food allergen is the major allergen in apple, Mal d 1. Because of its lability, apple extracts can rapidly lose their IgE-binding capacity after extraction.¹ The same is true for homologous allergens in many other fruit extracts, such as peach, cherry, and pear. Here, however, we want to focus on the opposite problem: false-positive in vitro test responses for specific IgE. In this issue, Mari et al² describe a special case of false-positive test responses caused by IgE antibodies directed to *cross-reactive carbohydrate determinants* (CCDs).

IgE antibodies against CCDs were first reported in the early 1980s.³ For pollen- and venom-allergic subjects, it was shown that these CCD structures are highly cross-reactive, resulting in IgE reactivity against a broad range of foods from plant and/or invertebrate animal origin. The responsible cross-reactive structure was concluded to be of carbohydrate nature because the IgE-binding epitope was destroyed by periodate treatment. Further support for the carbohydrate nature of CCDs came from the observation that the interaction is insensitive to heating and protease digestion of allergen extracts. Over the past decade, the structural basis of IgE binding to plant-derived carbohydrate epitopes was at least partly elucidated.⁴ Two typical nonmammalian substitutions to N-glycans of plant glycoproteins were shown to be involved: an $\alpha(1,3)$ -linked fucose on the proximal N-acetyl glucosamine and a $\beta(1,2)$ -linked xylose on the core mannose.

Patients allergic to pollen who demonstrate IgE reactivity with vegetable foods exclusively based on cross-reactive anti-CCD IgE were reported to be free of clinical food allergy. Recently, Van der Veen et al⁵ demonstrated that about 20% of subjects allergic to grass pollen

Abbreviations used

CCD: Cross-reactive carbohydrate determinant

SPT: Skin prick test

have false-positive responses to in vitro tests for peanut because of cross-reactive IgE antibodies against plant-derived carbohydrate determinants. Now Mari et al² once more focus our attention on the lack of biologic activity of carbohydrate-specific IgE antibodies. In their study they present a patient with significant serum IgE titers against several pollen species (eg, birch, mugwort, and olive) not accompanied by positive skin prick test (SPT) responses or by symptoms during pollination. Only grass and pellitory pollen, against which much higher IgE titers were found, induced positive SPT responses and hay fever. IgE binding to clinically irrelevant pollen species was inhibited with bromelain, a glycoprotein with a xylose/fucose-substituted N-glycan, and was sensitive to periodate treatment of the pollen extract. The frequency of anti-CCD IgE among subjects allergic to pollen was determined by performing SPTs and in vitro IgE measurements with bromelain. In approximately 40% of the subjects, bromelain-reactive IgE was detected. In all cases the SPT response was negative. This study convincingly demonstrates the relevance of anti-CCD IgE for the problem of false-positive in vitro allergy test responses.

Why are these IgE antibodies biologically inactive? Mari et al do not speculate about possible explanations. A type I allergic response is triggered by optimal cross-linking of IgE molecules that occupy high-affinity IgE receptors on mast cells. Possibly, IgE antibodies against carbohydrate determinants are not capable of cross-linking receptors. Cross-linking requires at least 2 epitopes on an allergen. Many glycoproteins in the molecular weight range of allergens (approximately 10 to 70 kD) are monoglycosylated. Bromelain is a good example. If IgE recognition is exclusively based on recognition of CCDs, cross-linking is not to be expected in this example. It is therefore not a surprise that bromelain fails to induce skin reactions. Preliminary data from our laboratory indicate, however, that even a glycoprotein with multiple N-glycans, such as horseradish peroxidase, cannot induce histamine release from human basophils that are sensitized with anti-CCD IgE. Confirmation of these observations would rule out epitope monovalency as an explanation for the lack of biologic activity of anti-CCD IgE. An alternative explanation could be the affinity of the interaction between IgE and glycans. This is currently being investigated by means of Scatchard analysis.

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Explaining why IgE anti-CCD is biologically inactive has a broader scope; specific IgE without clinical symptoms is a more general phenomenon. Not all patients allergic to pollen with cross-reactive IgE antibodies against the panallergen profilin⁶ have food allergy related to food profilins, even if their IgE recognizes these molecules. Similarly, recognition of Bet v 1 and its homologue in apple (Mal d 1) does not guarantee a clinical apple allergy.⁷

Can we improve in vitro diagnostic tests for specific IgE on the basis of the observation that IgE against glycans is clinically irrelevant? It is obvious that detection of biologically inactive IgE should be avoided. In particular, in vitro tests for food allergy could be improved in this way. In our laboratory we are currently testing the possibility of adding a soluble inhibitor during the serum incubation step of a RAST assay. Protease-digested grass pollen extract is used to prevent binding of serum IgE antibodies to plant-derived glycans of solid phase-immobilized allergen extracts. In particular, food allergy diagnostics will gain clinical relevance from this adjustment of current tests.

Over the past decade, many major allergens have been cloned and expressed as recombinant proteins. Expression of glycoproteins in *Escherichia coli* results in nonglycosylated proteins. Application of such nonglycosylated "glycoproteins" in allergy diagnostics is an alternative way to avoid detection of anti-CCD IgE. In some cases the lack of posttranslational modifications prevents correct folding of the recombinant protein, resulting in decreased IgE binding.⁸ Then, eukaryotic expression systems such as yeasts (*Saccharomyces cerevisiae* or *Pichia pastoris*) can prove to be useful. These organisms are able to glycosylate, but they lack the enzymes that substitute complex glycans with the typical plant features. Still, it remains to be investigated whether yeast-derived N-glycans are not recognized by IgE antibodies as well.

In conclusion, a better understanding of the role of IgE antibodies against CCDs is likely to improve the quality of in vitro diagnostic tests for allergy, particularly food allergy. The report by Mari et al² contributes to this understanding.

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